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16. ABSTRACT

Epidemiologic studies have suggested factors in drinking water influence on the human cardiovascular system. A clear identification of the factors involved requires more invasive techniques and more strict experimental controls than can usually be applied in epidemiologic studies. Consequently laboratory animals are often used to expand and support epidemiologic data. For laboratory purposes cardiovascular toxicology must be broken down to effects on the myocardium, the vasculature and the kidney. Further division may be necessary to take into account the influence of the neuroendocrine system or other systems that influence the function of the cardiovascular system. Since environmental influences upon the cardiovascular disease are relatively subtle (versus the acute effects of some drugs) it is assumed that major difficulties are chronic in nature. Accordingly, it is suggested that laboratory experimentation focus on either chronic toxin exposure or short-term exposure to stressed or genetically susceptible animals. A variety of in vivo and in vitro tests may be necessary to relate the toxicity realized in animals to what might be expected in man. Wide species differences with respect to the susceptibility of particular target tissues must be taken into account to clearly apply the results to man. Similarly, special characteristics of stressed and genetically susceptible animals must be understood to avoid deceptive results.

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EXPERIMENTAL APPROACHES TO EVALUATING THE ROLE OF ENVIRONMENTAL FACTORS IN THE DEVELOPMENT OF CARDIOVASCULAR DISEASE

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Epidemiologic studies have suggested factors in drinking water influence on the human cardiovascular system. A clear identification of the factors involved requires more invasive techniques and more strict experimental controls than can usually be applied in epidemiologic studies. Consequently laboratory animals are often used to expand and support epidemiologic data. For laboratory purposes cardiovascular toxicology must be broken down to effects on the myocardium, the vasculature and the kidney. Further division may be necessary to take into account the influence of the neuroendocrine system or other systems that influence the function of the cardiovascular system.

Since environmental influences upon the cardiovascular disease are relatively subtle (versus the acute effects of some drugs) it is assumed that major difficulties are chronic in nature. Accordingly, it is suggested that laboratory experimentation focus on either chronic toxin exposure or short-term exposure to stressed or genetically susceptible animals. A variety of in vivo and in vitro tests may be necessary to relate the toxicity realized in animals to what might be expected in man. Wide species differences with respect to the susceptibility of particular target tissues must be taken into account to clearly apply the results to man. Similarly, special characteristics of stressed and genetically susceptible animals must be understood to avoid deceptive results.

INTRODUCTION

There is little doubt that environmental factors do play a role in the development of human cardiovascular diseases. The epidemiological literature associating increased cardiovascular morbidity and mortality with a variety of risk factors is voluminous. However, it is notable that in addition to the

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usual dietary, genetic and socio-economic factors a small body of data does suggest that exposure to chemicals can play a role in the development of cardiovascular diseases. Most prominent is the relationship between cigarette smoking and coronary artery disease (Stamler, 1971; Kannel and Gordon, 1970) which can be shown to exhibit a clear dose-response relationship (Miettinen *et al.*, 1976).

The conclusions of the review by Sharrett and Feinleib (1975) concerning the negative correlation between hardness of drinking water and cardiovascular disease still stand today. The finding of such relationships is reasonably consistent in large national studies. However, the tendency for weaker relationships have been found when studied in smaller regions. Often neglected in the national studies is the fact that the hardness of drinking water varies regionally (i.e., coastal areas have softer water and high plains hard water). Consequently the relationship between water hardness may in fact be confounded by regional variables not even related to water quality. Furthermore, conflicting results have been derived when such studies have attempted to focus on single elements in drinking water or particular types of cardiovascular disease.

One means of resolving problems of this complexity has classically been through animal studies. Although animal studies do not always provide comprehensive models for human disease processes, at the minimum they can provide substantial support for a particular conclusion reached in the population studies. The ability to control the critical variables in experimental animals can provide for definitive conclusions within the context of the experiment. If such results can be shown to be consistent with the disease state as it is known in humans a strong argument can be made for regulating the environmental circumstances suggested by epidemiological data to be responsible.

In general, there is good agreement between the influence of risk factors in human cardiovascular diseases and that observed in experimental animals. On the other hand, there are genuine pitfalls in assuming that results in experimental animals can be transferred to humans casually. Species peculiarities mark to a greater or lesser degree the appropriateness of a particular result to the human situation. The purpose of this paper is to call attention to some of these problems and hopefully provide a critical framework for future work in environmentally-induced cardiovascular disease.

ENVIRONMENTAL CHEMICALS WHICH PRODUCE CARDIOVASCULAR TOXICITY

Since this symposium is considering water factors and cardiovascular disease, this section will emphasize chemicals which have been found in drinking water. Certain other chemicals which are of general environmental interest will also be included. Except for specific model compounds, the large body of data concerning drug toxicities to the cardiovascular system will not be considered.

Hard and Soft Water and Cardiovascular Diseases

The primary evidence for the role of drinking water in cardiovascular disease is the negative correlation between water hardness and cardiovascular mortality first reported by Schroeder (1960 a, b) and reviewed by Sharrett and Feinleib (1975). Water hardness is not a precisely defined chemical characteristics of water. Rather it is an empirical formulation primarily referrable to the multivalent cation content of water. Conversely, softness of water refers to a relatively low content of multivalent cations. The major cations making up hardness are Ca^{++} (range approximately 1 - 150 mg/1) and Mg^{++} (1 - 30 mg/1). Generally lesser constituents in approximate descending order of concentration and occurrence are Sr^{++}, Fe^{++}, Al^{+++} and Mn^{++}.

An important consideration is that soft water is generally much more corrosive than hard water. Metals used in the construction and installation of water mains and pipes tend to be leached into the water by soft, acidic waters (Craun and McCabe, 1975).

As a consequence of these considerations there are three possible general hypotheses to explain the negative correlation between water hardness and cardiovascular disease mortality:

1. Presence of essential trace metal(s) in hard water protects against cardiovascular disease
2. Presence of toxic trace metal(s) in soft water that is causally related to cardiovascular disease
3. The relationship between water hardness and cardiovascular disease is spurious and merely covaries with non-metallic contaminants in drinking water

There is little information available from controlled animal experiments that lend any definitive support to any one of these hypotheses. However, data available in the general literature does lend support to each of these alternate hypotheses. The following is meant to be illustrative of the area rather than a definitive review of the relevant literature. It is expected that specific points will be pursued more in depth by other authors in this symposium.

The contribution of hard drinking water to the nutritional requirement for calcium and magnesium has been shown to be small compared to dietary intake (Hankin *et al.*, 1970). However, if it is assumed that intake of these ions was marginal in the diet of substantial segments of the American population (Seelig and Heggtveit, 1974; McBean and Speckmann, 1974) the relatively small contribution available from hard drinking water may be disproportionately important.

Magnesium deficiency has long been associated with pathology of the cardiovascular system (Seelig and Heggveit, 1974). Cardiac necrosis and calcification are observed within 14 days of placing rats on a Mg^{++} deficient diet (Heggtveit *et al.*, 1964). As early as 1933, Krause *et al.*, noted that magnesium deficiency in dogs produced elevations in blood lipids, including cholesterol esters. Experiments conducted by Vitale and coworkers (Vitale *et al.*, 1957; Hellerstein *et al.*, 1957) indicated that magnesium administered to rats on a high fat diet decreased the development of cardiac and renal and atherosc-

lerotic lesions (defined by Sundanophilia). Neal and Neal (1962) indicated prevention of atherosclerotic lesions in rats with very high doses of magnesium. In total there is considerable data that can be taken as supportive of the idea that Mg is the protective factor present in drinking water. Unfortunately each of these studies possess serious methodological problems with respect to the relatively small contribution hard water could make to total magnesium intake. Consequently, magnesium's role as the factor present in hard water that protects against cardiovascular disease is yet to be established under conditions more closely approximating environmental conditions.

The role of calcium from drinking water as a protective factor in cardiovascular disease is even less clear. There is no doubt that calcium plays a variety of critical roles in the normal function of the cardiovascular system. Its involvement in excitation-contraction coupling, cellular energetics and as a "second messenger" for neuroendocrine influences on cardiovascular function is well established (Fleckstein, 1977). At the cellular level there is substantial evidence for several roles of calcium in heart failure (Dhalla, 1976). Increases in calcium concentrations produced *in vitro* or by *in situ* perfusion are clearly related to vasoconstriction (Maxwell *et al.*, 1964) increased cardiac work, oxygen consumption, and pacemaker activity (Fleckstein, 1977). These effects are not clearly relatable to a protective effect against the development of cardiovascular disease. In addition, there is no data which demonstrates that even large excursions in calcium intake (above the minimum daily requirement) significantly alters serum or tissue calcium concentrations. Since serum calcium concentrations are closely controlled by complex feedback mechanisms, this is perhaps not surprising.

Fleischmann and coworkers (Fleischmann *et al.*, 1966, 1967; Yacowitz *et al.*, 1965) demonstrated that very high levels of dietary calcium decreased serum lipid and increased fecal lipids. Many investigators, however, hold that this effect is non-specific involving the ability of divalent cations to form poorly absorbed salts with saturated fatty acids (Speckman and Brink, 1967). Basically it is not at all apparent what impact suboptimal calcium intakes might have on cardiovascular function simply because of a lack of data bearing on the issue. As with magnesium it is not possible to speculate profitably as to what benefits might arise in the context of the relatively small (<50% of the MDR) portion of Ca^{++} that might be derived from hard water.

Toxic factors found in soft water that *might* account for increased cardiovascular diseases could be used to construct a very large list (McCabe *et al.*, 1970; David and DeWiest, 1966). If kept within limits of probability (for present purposes a function of concentration encountered) and the properties of soft water, attention focuses on trace metals which might leach from drinking water distribution systems (Schroeder and Kraemer, 1974). The principle metal which has been demonstrated to be leached from distribution systems is Pb (Craun and McCabe, 1975; Greathouse *et al.*, 1976). A second candidate, Cd, has yet to be demonstrated as a consistent contaminant of soft water.

Lead exposure has been established as a chemical which can induce chronic nephropathy in humans (Cramer *et al.*, 1974) as well as experimental animals (Goyer, 1968). Such damage has been exclusively associated with

relatively high exposures to Pb, however. Virtually no data exists that would demonstrate the occurrence of effects on the kidney at blood concentrations approaching the upper range of normal for non-occupationally exposed humans. Blood and tap-water Pb concentrations have been found to be slightly elevated in hypertensive vs. normotensives in Scotland (Beevers *et al.*, 1976). An earlier study of a large occupationally exposed population (blood Pb > 80 mg/dl) also revealed excess mortality associated with chronic nephritis and hypertension (Tabershaw and Cooper, 1974). Chronic Pb nephropathy is associated with pronounced arteriosclerotic changes in the kidney (Cramer *et al.*, 1974). However, animal experiments concerning the the cardiovascular toxicity of Pb appear to be limited to ultrastructural changes in the myocardium observed at relatively high blood Ph concentrations (Asokan, 1974). A study by Moore *et al.*, (1975) indicates a similar, but reversible picture at very low doses of Pb (i.e. 1 mg/1 of drinking water). In an extremely interesting study Ph administered to dams resulted in a sensitization of the myocardium to norepinephrine epinephrine induced cardiac arrythmias in rat pups (Williams *et al.*, 1977a). Although it certainly cannot be said that from these studies that Pb is the "water factor" the limited data available indicates that it must be considered a candidate.

As cadmium is the subject of other presentations at this symposium, attention is merely called to its established effects on the kidney and its suggested role in hypertension (Kanisawa and Schroeder, 1969). Other inorganic constituents of drinking water known capable of influencing the cardiovascular system include Ba, Cr, As, V and Li. A tabulation of the principle benefits and/or abnormalities associated with these elements appears in Table 1.

Halogenated hydrocarbons are major contaminants of drinking water. Many compounds of this class have been shown to affect cardiovascular function. These compounds appear in drinking water as industrial and/or municipal pollutants and by the reaction of organic chemicals in the source water with chlorine used in disinfection. As a consequence, one could argue that these chemicals would be more common in surface water sources (which would tend towards softer water) than in groundwater (tending towards hardness in certain geographic areas). There would be numerous exceptions to this, but the generalization is sufficiently accurate to prevent exclusion of the possibility that such chemicals might account for differences in cardiovascular mortality.

Investigation of the cardiovascular toxicity of the halogenated hydrocarbons has been substantially limited to high dose acute exposures. The use of these compounds as general anesthetics and the very high level exposures which result from their use industrially accounts for the focus on acute exposures in the scientific literature.

Chloroform is the most common halogenated hydrocarbon in drinking water. Chloroform at anesthetic concentrations produces myocardial depression, ventricular ecotopic beats and increases the ability of epinephrine to induce ventricular arrythmias (Waters, ed., 1950). Similar findings have been made with other low molecular weight chlorinated hydrocarbons including

TABLE 1. Elements Associated with Drinking Water Postulated as Having a Role in Preventing or Promoting Cardiovascular Disease

Element	Species	Relevant Biological Property	References
Calcium	various species	Modifies lipid absorption. Essential in excitation contraction of myocardium and smooth muscle.	Speckmann & Brink (1967)
		Essential neuroendocrine function.	Fleckstein (1977)
		Modifier of lipid metabolism	
		Vasoconstrictor	Maxwell *et al.* (1964)
Magnesium	various species	Essential in cellular energetics.	
		Suppress cardiac, renal and vascular lesions of deficiency.	Hellerstein *et al.* (1957)
	dog	Vasodilator	Maxwell *et al.* (1963)
Barium	human and various species	Cardiac arrythmias.	Roza and Berman (1971)
		Positive inotropic effect.	Higgins *et al.* (1976)
			Foster *et al.* (1977)
Lead	human and various species	Chronic nephropathy with renal atherosclerotic changes.	Cramer *et al.* (1974)
		Hypertension	Beevers *et al.* (1976), Asokan (1974)
	rat	Myocardial damage	Asokan (1974)
	rat	Developmental sensitization to epinephrine-induced arrythmias.	Williams *et al.* (1977a)
Cadmium	rat	Hypertension	Schroeder (1964)
	human, rat, rabbit	Renal lesions	Kanisawa & Schroeder (1969)
Chromium	human & rat	Glucose tolerance factor	Gurson & Saner (1971)
			Schwarz & Mertz (1959)
	rat	Lowers serum cholesterol	Mertz (1969)
	rat	Deficiency results in aortic plaques	Schroeder & Balassa (1963)
Arsenic	human	Renal damage	
		Capillary damage	Zaldivar & Guiller (1977)
		Fibrous intimal thickening of arteries.	
		Cardiac arrythmias	Butzengeiger (1949)
Vanadium	various	Positive inotropic effect	Grupp *et al.* (1979)
		Inhibits hepatic synthesis of cholesterol	Curran (1954)
Lithium	human	Cardiac arrythmia	Hansen & Amdisen (1978)
		Chronic nephropathy	

trichloroethylene (Defalque, 1961) 1,1,1-trichloroethane (Reinhart *et al.*, 1973) and 1,1-dichloroethylene (Siletchnik and Carlson, 1974). Findings are basically consistent between man and experimental animals and extend to fluorinated hydrocarbons not generally found in drinking water (Aviado, 1978).

In the case of 1,1-dichloroethylene evidence was obtained suggesting that a metabolite of 1,1-DCE rather than the parent compound was responsible for the cardiotoxicity (Siletchnik and Carlson, 1974). On the other hand, the sensitization of the myocardium to epinephrine by trichloroethylene appeared to be decreased by stimulated metabolism of the compound (White and Carlson, 1979). Despite the closely related nature and metabolism of these two compounds it would appear that generalizations based on chemical structure and metabolic pathway are not possible.

Chronic cardiovascular effects of the chlorinated hydrocarbons does not seem to have been pursued experimentally in any depth. Epidemiological data does suggest, however, that the chlorinated ethylenes (Grandjean *et al.*, 1955) do produce some longer-term neurological and "vegetative" disturbances in occupationally exposed groups. In controlled human studies, the data of Stewart *et al.* (1961) showed a consistent increase in the blood pressure of volunteers exposed to tetrachloroethylene by inhalation, for 5 consecutive days although the result was not discussed in the paper. Vinyl chloride, or monochloroethylene, has been associated with portal hypertension (Kurokawa *et al.*, 1977; Smith *et al.*, 1976) that appears more commonly in workers than angiosarcoma. Additionally, vinyl chloride is known to produce severe damage to the peripheral vasculature (Williams *et al.*, 1977b). Similar pathology is observed in mice chronically ingesting the chlorinated pesticides, aldrin, dieldrin, heptachlor or heptachlor epoxide (Reuber, 1977).

Carbon disulfide was originally recognized as a neurotoxin. Only recently has it been recognized that levels of carbon disulfide below those established on neurotoxicological considerations, have resulted in increased mortality from coronary heart disease (Tiller *et al.*, 1968; Partanen *et al.*, 1970). Oddly enough experiments in a variety of experimental animals had predicted the atherogenic nature of carbon disulfide some 20 years before it was recognized in humans (Davidson and Feinleib, 1972).

Although they are not chemicals of general environmental concern, the anthracycline antibiotics, daunorubicin and dixorubicin (Adriamycin), serve as examples of direct myocardial toxins. The cardiomyopathy observed, which involved myofibrils, intracellular junction, T tubules, sacroplasmic reticulum and mitochondria, is quite similar in humans, rats, mice and rabbits (Balazs and Ferrans, 1978). This further strengthens the qualitative, if not quantitative, reliance one can place on properly conducted animal experiments.

As a final note to this section, attention should be called to the work of Albert *et al.* (1977) which demonstrated accelerated development of atherosclerotic plaques in chickens by the carcinogens dimethylbenzanthracene and benzo(a)pyrene. This experiment was initiated as a test of the hypothesis that atherosclerotic lesions are benign smooth muscle tumors. This hypothesis arose from the suggestion atherosclerotic plaques were monoclonal in origin

(Benditt and Benditt, 1973). Although this experiment does not prove this hypothesis, it does suggest that atherosclerosis can be produced by a broader range of chemical substances than has been previously appreciated.

CARDIOVASCULAR DISEASES AND ANIMAL MODELS

Atherosclerosis

Atherosclerosis is the most lethal form of arteriosclerosis or hardening of the arteries. It underlies most forms of ischemic heart disease, and plays a major role in cerebrovascular disease and hypertension. Indoing so atherosclerosis becomes the leading single cause of death in the United States (Frederickson, 1974).

As defined in the 23rd Edition of Stedman's Medical Dictionary atherosclerosis is "Nodular sclerosis; arteriosclerosis characterized by lipid deposits in the intima, irregular distribution in large and medium sized arteries. The lipid deposits are associated with fibrosis and calcification....etc." Induction of this lesion seems to be first associated with endothelial cell damage or discontinuity. This damage may be caused by physical means for example hemodynamic stress points in arteries or chemical means (cigarette smoke) (Vesselinovitch, 1979; Ross *et al.*, 1977; Ross and Harker, 1976; Thorgiersson and Robertson, 1978; Stemerman *et al.*, 1977). Endothelial damage is followed by platelet adherance. In major and intermediate sized arteries, platelet adherance is associated with microthrombi, mitogen secretion and smooth muscle outgrowth and or migration from deeper layers. If serum lipid levels are very low this may result in a scarring or arteriosclerotic plaque formation. However when serum lipid levels are high, lipids are incorporated into the lesion and it may eventually grow into an atherosclerotic plaque as earlier defined (Stemerman *et al.*, 1977; Rose and Glomset, 1973; Ross *et al.*, 1977).

The importance of the lipid inclusion and serum lipid levels are pointed out by the fact that in human populations with mean serum cholesterol levels of 160 mg% and below there is little evidence of symptomatic atherosclerosis (Goldstein and Brown, 1977).

Most serum cholesterol is found in lipoprotein vesicles with phospholipids and triglycerides (reviewed by Jackson *et al.*, 1976; Goldstein and Brown, 1977). These lipoproteins exist in at least five distinct forms in normal humans. The nomenclature has been assigned by centrifuged sedimentation density or electrophoretic mobility. They are chylomicrons (< 0.95 g/ml or origin); very low density lipoprotein (VLDL) (0.95 - 1.006 g/ml or prebeta); low density lipoprotein (LDL) (1.006 1.063 g/ml or beta); high density lipoprotein (HDL) (1.063 - 1.210 g/ml or alpha); and very high density lipoprotein (VHDL) (1.210 - 1.250 g/ml or alpha).

Chylomicrons are synthesized in intestine and transported through lymph ducts to general circulation. They are taken up by liver and converted to VLDL and that is converted to LDL in circulation by losing triglycerides to fatty tissue and accepting cholesterol from HDL. HDL is synthesized in liver and intestine (Jackson *et al.*, 1976).

Tissue cholesterol levels are regulated by a receptor mediated pinocytotic uptake mechanism accepting LDL vesicles. The cholesterol from the absorbed LDL regulates intracellular cholesterol levels by (1) inhibiting the rate limiting enzyme in cellular cholesterol synthesis, (2) stimulating cholesterol esterification and consequently cholesterol storage (3) inhibiting synthesis of LDL receptor protein and thus inhibiting the uptake of more cholesterol (Brown and Goldstein, 1979). In hyperlipidemia the receptor uptake mechanism is in some way over-ridden or subverted in such a way that cholesterol may be accumulated at pathologic levels. When this happens in smooth muscle cells as a step in the process outlined earlier, atherosclerosis results. It should be noted that hyperlipidimia may be the result of a defect in LDL receptor system. In some types of familial hypercholesterolemia increased serum LDL compensates for a decreased ability to bind and internalize LDL (Brown and Goldstein, 1979).

A clear understanding of the function of HDL is not to be had at this time, but high levels of HDL especially HDL$_2$ are associated with decreased incidence of atherosclerosis and myocardial infarction (Miller, 1978; Bank *et al.*, 1971; Marx, 1979; Barboriak *et al.*, 1979). It has been suggested that HDL removes excess cholesterol from cells and transports it to liver for catabolism and excretion, or in some way interferes with excess cholesterol uptake from LDL to cells (Marx, 1979).

Clearly the development of atherosclerosis is a multistep process. There are several points in this process where environmental toxins exacerbate, initiate or amiliorate a pathologic condition. Some examples of chemical intervention include cigarette smoking decreasing serum HDL levels (Wood and Haskell, 1979) while alcohol ingestion increases serum HDL (Barboriak *et al.*, 1979). A similar effect can be seen with ingestion of sex hormones in birth control pills. Progestins decrease and estrogens increase serum HDL (Krauss *et al.*, 1979). Aspirin has been shown to abolish platlet adherence to endothelial damaged arteries (Ratcliff *et al.*, 1979) and homocysteine (Harker *et al.*, 1974) and hypercholesterolemia (Gerrity and Schwrtz, 1977) have been shown to damage vascular endothelium. Recently, it has been suggested that atherosclerotic plaques are essentially benign tumors of vascular smooth muscle tissue (Benditt, 1974). Although this suggestion has been challenged (Thomas *et al.*, 1977 and Pearson *et al.*, 1979), the carcinogens benzo(a)pyrene and dimethylbenz(a,h)anthracene have been found to increase the frequency and size of atherosclerotic plaques (Albert *et al.*, 1977).

The use of animal models for human atherosclerosis has contributed considerably to the understanding of this disease. However, caution must be used in extrapolating animal data to humans particularly in the case of atherosclerosis, since humans are one of the most sensitive species of this disease. In fact, the most commonly used laboratory species do not develop atherosclerosis under normal laboratory conditions. As a result, many animal species relatively uncommon in laboratory research may be better suited to study atherosclerosiis.

Some characteristics which an ideal laboratory model should include: (1) It should have dietary characteristics similar to humans, since diet and metabo-

lism of diet in humans has been established as an atherosclerotic risk factor. (2) It should develop atherosclerosis in vital arteries of the heart and brain as well as in the aorta. (3) It should develop complications of atherosclerosis such as stroke, hypertension and myocardial infarction. (4) It should be small, cheap and easy to handle and house for studies involving large numbers of animals and it should be large enough to facilitate morphologic observation of arteries and simple surgery. (5) It should have functionally,structurally and biochemically similar arteries and heart to those found in man. No animal fits all of these criteria. Consequently, studying the influence of toxic chemicals on the development of atherosclerosis will require careful evaluation of alternate animal models to determine that which is best suited.

Table 2 lists some of the most commonly used animal models with some advantages and disadvantages of each. Of those listed only the rat, rabbit and some non-human primates do not develop spontaneous atherosclerotic lesions (except very late in life and even then in very minor proportions). Given a 1% cholesterol diet however, Dutch Belt rabbit serum cholesterol increases from 75 mg/dl to 880 mg/dl and New Zealand white rabbit from 80 mg/dl to 1640 mg/dl (Shore and Shore, 1975). This type of manipulation of serum lipids is associated in rabbit with atherosclerotic like fatty lesions without a fibrosis cap and calcification (Kritchevsky *et al.*, 1976). Further manipulation of diet (sporadic high cholesterol) may induce these last two characteristics of atherosclerosis (Constantinides *et al.*, 1960). Other mammals in which diet-induced atherosclerosis is easily produced include lemmings, voles (Dieterich and Preston, 1979) and diabetic and non-diabetic hamsters (Soret *et al.*, 1975). As in the case of the rabbit, they are herbivorous and develop fatty livers as well as fatty aorta in response to hypercholestrolemia.

Common laboratory animals which are omnivorous or carnivorous such as rat and dog are resistent to diet-induced hypercholesterolemia. However, if a diet including fats and cholate is administered to thyroid gland impaired rats or dogs hyperlipidemia, atherosclerosis and thrombic myocardial infarction will result. Thyroid gland impairment may be caused by throid ablation in dogs (DePalma *et al.*, 1972) or with goiterogenic agents such as propylthiouracil in rats (Thomas and Hartroft, 1959; Thomas *et al.*, 1963). Thyroid hormone enhances the ability of the liver to secrete cholesterol in bile and consequently hypothroidism causes serum hyperlipidemia (Goodman and Middelworth, 1974; Story *et al.*, 1974). Thyroid insufficiency also causes increased cholesterol infiltration of the liver (Thomas *et al.*, 1963).

Other approaches used to induce hypercholesterolemia and atherosclerosis in diet-induced hypercholesterolemic resistent animals include use of genetic mutant models such as the Zucker-Koletsky rat (Zucker and Zucker, 1961; Koletsky, 1973). (The Zucker and Koletsky rats are apparently identical mutants (Yen *et al.*, 1977) both obese, hypertensive and hypercholesterolemic). Selective breeding for dietary susceptibility to hyperlipidemia has been successful in mice (Roberts and Thompson, 1975) and for spontaneous hypercholesterolemia in rats (Iami and Matsumura, 1973; Muller *et al.*, 1979).

The animal models in the preceeding discussion under normal conditions have serum cholesterol levels below 100 mg/dl (see Table 3). In order to induce

TABLE 2. Some Species Used in Laboratory Studies of Atherosclerosis

Human	Advantages — Obvious. Disadvantages — Severely limited *in vivo* experimentation. Difficulty of acquiring tissue for *in vitro* analysis.
Rabbit	Advantages — Well characterized physiology and pathology, ease of handling, low expense. Disadvantages — Low rate of and usual atypical spontaneous lesion develop. Herbivorous. Manipulations needed to develop atherosclerotic plaque differ from other species. Hyperlipidemic models demonstrate unusual composition of lipoproteins.
Rat	Advantages — Small, well characterized. Many different genetic Hyper-cholesterolemic strains. Disadvantages — Usual lab strains do not develop spontaneous atherosclerosis. Difficult to induce by diet. Major structural differences in aorta. (1) No Vasa Vasorum. (2) Extremely thin intimal layer. Extremely hypolipidemic. Ratio of cholesterol in LDL to HDL very low.
Chicken	Advantages — Spontaneous atherosclerosis. Similar serum cholesterol levels to man. Easily inducible lesion and hyperlipidemia. Genetic hyperlipidemic models available. Atherosclerosis may develop into arterial stenosis. Omnivorous. Disadvantages — Limited distribution of lesions. Considerable small artery involvement. Avian.
Pigeon	Advantages — Spontaneous atherosclerosis resembling that of humans in both morphology and distribution. Atherosclerotic resistant and prone breeds. Genetic hypercholesteremic variants. Atherosclerotic plaques do eventually obstruct lumen. Myocardial infarcts have been reported. Disadvantages — Foam cell cerived from smooth muscle only. Herbivorus. Avian.
Swine	Advantages — Spontaneous atherosclerosis. Omnivorous with similar aorta and coronary artery structure. Spontaneous atherosclerosis of similar type and distribution as in humans. Atherosclerotic resistant genetic varient (von Willebrands disease) known. Disadvantages — Large size and difficulty of handling. Myocardial infarction and sudden death only reported after severe physical coronary artery injury. 6-12 years necessary to develop atherosclerotic lesions but may be sped u with diet or diet plus artery injury.
Non-human primates	Advantages — Omnivorous in most cases. Many species and genetic variants available with a variety of characteristics pertinant to atherosclerosis. Closest species phylogenetically to man. Disadvantages — Difficult to handle. Prone to stress related complications. May acquire or transmit human diseases. Difficult to procure and house in large numbers necessary for large studies.

atherosclerosis in these animals serum cholesterol must be raised (the minimum borderline levels range from 150-200 mg/dl). Therefore, all of these animals when induced (by diet or genetics) suffer from unnatural hyperlipidemia. In every case discussed, hyperlipidemia results in more pathology than just atherosclerosis. All of the animals had at least fatty livers except one strain of rat that suffered from glomerulonephritis. Either one of these pathologies (liver or kidney) might inhibit toxin metabolism and/or excretion. However, the major difficulty would be that the effects of a toxin which acted through lipid metabolism might well be obscured by the already elevated and/or modified lipids found in these animal models. Furthermore, in animals such as the rat, that require thyroid insufficiency, as well as dietary fats and cholate in order to develop hyperlipidemia, atherosclerosis and consequent myocardial infarction the multiple pathology problem is exacerbated. One might wonder if this myocardial infarction requires hypothyroidism or are the cardiovascular effects of hypothyroidism and diet limited to serum cholesterol regulation.

The species listed in Table 3 that develop spontaneous atherosclerosis are humans, swine, chicken, Japanese Sea Gull, and White Carneau pigeon. General characteristics of susceptable mammalian species are high serum cholesterol, high serum LDL cholesterol and high LDL/HDL cholesterol ratio. The importance of the LDL/HDL ratio may best be seen by comparing coronary artery patients' ratio to that of the general public. An increase in LDL/HDL ratio even within species that do not ordinarily develop atherosclerosis might well be predictive of problems in a species susceptible to atherosclerosis (Müller and Cortesi, 1978). On the other hand, an environmental agent which increases total serum cholesterol by increasing serum HDL may well be beneficial to the cardiovascular system (Barboriak *et al.*, 1979) and one which decreases serum cholesterol by decreasing HDL may be detrimental (Day *et al.*, 1979; Bondjers *et al.*, 1976).

Swine are well suited for studies of atherosclerosis for several reasons; (1) they are omnivorous, (2) the structure of the aorta and coronary arteries are similar to that of man, (3) atherosclerosis begins early in life as in human and (4) they develop arterial lesions in aorta, coronary and cerebral arteries similar to that of humans without special diets (Wissler and Vasselinovetch, 1977; Florentein *et al.*, 1968; Lugenbuhl *et al.*, 1979; Taura *eet al.*, 1978). Swine like man however, take years (3-12 in the swine) to develop these lesions. Several investigators induced earlier lesion formation by high cholesterol diet (Florentein *et al.*, 1968) or intimal injury and high cholesterol diet (Daoud *et al.*, 1976). Pre-proliferative endothelial changes have been demonstrated as early as 6 weeks after initiation of cholesterol diet with lard (Gerrity and Schwartz, 1977). One must, however, be cautious of such measures for reasons already discussed.

Although several spontaneous lesions have been reported in swine, the incidence of spontaneous myocardial infarction is uncommon or absent but has been induced by massive X-radiation delivered to the base of the heart (Lee *et al.*, 1970) and by a combinationof balloon catheter endothelial denudation of coronary arteries and high cholesterol diet (Lee and Lee, 1975).

TABLE 3. Plasma Cholesterol Distribution in Several Species

	Serum Cholesterol mg/dl	VLDL Cholesterol mg/dl	LDL[8] Cholesterol mg/dl	HDL Cholesterol mg/dl	HDL HDL Cholesterol
HUMAN					
Male[4]	207	13	137	57	2.40
Female[4]	206	15	122	69	1.77
Coronary artery[1] surgery patients.	—	40	162	41	3.95
SWINE[5,7]	124	4	67	52	1.26
Duroc[4]	93	2	73	17	4.29
Hamshire Gilt[4]	105	1	64	32	2.00
RAT[6,7]	62	8	4	50	0.08
Holtzman[2]	80	7	4	45	0.15
Norvegicus male[3]	52	7	7	34	0.21
Female[3]	60	4	6	52	0.12
Rico male[3]	107	4	11	97	0.11
Female[3]	151	4	14	133	0.11
RABBIT[6,7]	57	14	22	21	1.05
CAT[6,7]	78	2	18	58	0.31
MONKEY					
Cynomolgus Male[6]	127	2	57	67	0.85
Female[6]	125	3	58	64	0.90
CHICKEN					
New Hampshire Columbian Cross[2]	215	—	29	126	0.23
Japanese SEA GULL[6]	261	9	76	176	0.43
PIGEON, White					
Carneau[3]	211	—	59	130	0.45

[1]Nestel and Poyser, 1978
[2]Narayan and Calhoun, 1975
[3]Revis, Personal Commun.
[4]Glatz and Vislocky, 1979
[5]Muller *et al.*, 1979
[6]Day *et al.*, 1979
[7]Strain unidentified
[8]May include IDL

atherosclerotic lesions, and the placement of lesions within the vasculature. The reader is referred to recent reviews which admirably describe atherosclerotic disease in non-human primates in much greater detail than possible here (Clarkson *et al.*, 1976); (Lugenbuhl *et al.*, 1977; Wissler and Vesselinovitch, 1977; Vesselinovitch, 1979; Vesselinovitch and Wissler, 1975).

Pigeon and swine may be as interesting for their resistent substrains as they are for their spontaneous atherosclerosis. Swine with von Willenbrands disease (von Willebrands disease is associated with vastly decreased platelet adhesive qualities) develop considerably fewer and smaller atherosclerotic lesions than controls. However they do get fatty inclusion of the intima with no intimal thickening (Fuster *et al.*, 1975). A strain of Show Racer Pigeon develops no atherosclerotic lesions at 3 years of age in the intimal cushion of the celiac artery where another closely related strain of pigeon (White Carneau Pigeon) with the same diet, environment, serum triglycerides and serum lipid and lipid profile, develops 100% spontaneous lesions. The Show Racer Pigeon has a deficiency of platelet factor four (Fuster *et al.*, 1977). (Factor four demonstrates physical chemaical similarities (Lewis *et al.*, 1977) to Ross's mitogenic factor (Ross *et al.*, 1974)). It has been demonstrated that thrombocytes (analogous to platelets in pigeon) bind to damaged endothelia and its intimal cushion in White Carneau Pigeon (Lewis and Kottke, 1977) and in areas of increased endothelial permeability to Evans blue dye in swine aorta (Gerrity and Schwartz, 1977). From these two studies it can be concluded that at least in some cases platelet binding and secretion of mitogenic factors at sites of damaged endothelium promotes or initiates atherosclerosis. Environmental modification of platelet factor 4 activity is an area as yet untested and may be a useful area of investigation.

A general approach to determining the importance of environmental chemicals in the development of atherosclerosis must be cost-effective. Consequently considerations of time and space become very important. A reasonable approach would be to utilize smaller species in which the atheroscoerosis process might be accelerated. For this purpose studies in the pigeon, chicken or even the rabbit might be preferred. However such systems may give rise to artifactual results for various reasons, some of which have been mentioned above. Consequently confirmatory experiments in selected incidences should be conducted in mammalian species which develop spontaneous atherosclerosis without artifactual manipulations. For these purposes the swine might be preferred followed by non-human primates.

Hypertension

When a resting, sitting adult has arterial blood pressure of 160/95 or higher, that person is considered hypertensive. This hypertension if chronic in nature may usually be considered to be one of three types of hypertension, essential, renal or adrenal hypertension (Jagger and Braunwald, 1974).

Ninety percent of hypertension in the human population is of unknown cause, that is essential hypertension. It develops usually at maturity in individuals between 35 and 55 years of age. In some cases it may be associated with a decreased arteriolar or arterial size that may be due to vascoconstriction,

arteriosclerosis, or sodium induced artery wall thickening with concurrent lumenal shrinkage. There appears to be some genetic predisposition to essential hypertension. In some cases such as increased sodium intake, there appears to be synergism betwen genetics and environmental factors.

Chronic renal hypertension is caused by partial atherosclerotic occlusion of the renal artery resulting in consequent decreases renal perfusion pressure. A renal regulatory mechanism activates the renin angiotension pressor system which increases the systemic blood pressure. Angiotensin also causes the release of aldosterone with concurrent sodium and water retention. A second type of renal hypertension is associated with kidney parenchymal disease. It involves a capillary arteriosclerosis with glomerular involvement. This disease is often seen associated with Diabetes Mellitus or immune responses to environmental agents such as pollens, poison ivy and poison oak. It has also been suggested as a toxic manifestation of chronic industrial solvent exposures.

Adrenal hypertension is characterized by increased and often uncontrolled release of corticoids, usually aldosterone from the adrenal cortex. Aldosterone promotes retention of sodium and water. Since it inhibits release of renin, it is a relatively pure sodium loading hypertension. Even with increased aldosterone, hypertension may be avoided by simply decreasing sodium intake.

There are other forms for example, systolic hypertension caused primarily by aortic noncompliance due to arteriosclerosis or cardiac ventricular valvular insufficiency.

It should be noted that chronic hypertension exacerbates or actually causes other cardiovascular diseases, for example stroke and arteriosclerosis, myocardial hypertrophic ischemia, congestive heart failure or myocardial infarction. In fact certain forms of cardiovascular disease for example hypertension and arteriosclerosis, operate in a self-reinforcing cycle of mutual exacerbation.

Possibly the best animal to study hypertension is in the young rat. At this age the animal is particularly resistent to spontaneous arteriosclerosis. Since hypertension can in itself lead to other cardiovascular diseases and vice versa, it is important to identify agents which are purely hypertensive. Consequently, the rat can be used in conjunction with spontaneously atherosclerotic species such as the pigeon, as a means of distinguishing primary hypertensive agents from chemicals producing hypertension secondary to atherosclerosis.

A wide variety of hypertensive models exist in rats (Hallbach and Weiss, 1977). These rats may be used to model the effects of toxins in sensitive human populations. Among these models are:

1. Wistar Kyoto spontaneously hypertensive rat (SHR) (Okamoto and Aoki, 1963). This strain of rat develops extreme hypertension as it matures and may be equivalent of essential hypertension in humans (Yamori and Okamoto, 1973; Yamori, 1977; Fukiyama *et al.*, 1976).
2. Wistar Kyoto spontaneously hypertensive rat; stroke prone substrain (SHR-SP). This genetic varient develops cerebrovascular accident much earlier than the parent strain (Okamoto *et al.*, 1974; Yamori *et al.*,1976). When salt loaded it will develop renal lesions and cerebrovascular accident within five weeks (Shibota *et al.*, 1979).

3. Dahl Rat. This rat is a mutant with high susceptibility to sodium induced hypertension (Dahl *et al.*, 1962).
4. Adrenal hypertensive rat. Desoxy-corticosterone acetate (DOCA) implant mimics adrenal hypertension (Seyle *et al.*, 1943).
5. Glomerulonephritis model (SHC). This Sprague-Dawley substrain is hypercholesterolemic and develops spontaneous glomerulonephritis (Iami *et al.*, 1977).
6. Rena hypertensive model. Single renal artery partial ligation of rats induces rein mediated hypertension as in atherosclerotic partial occlusion of renal aretery (Rojo-Ortega, 1968).
7. Spontaneous, renal hypertensive rat (MHS) (Milan Strain). This rat develops non-renin mediated renal hypertension (Bianchi *et al.*, 1974, 1975).

The advantage of having all of these models available in one species especially a species as easy to handle as rat is considerable.

Myocardial Injury

The obvious dependence of the cardiovascular system on the effectiveness of the heart as a pump makes it essential that any consideration of cardiovascular toxicity include myocardial injury. Oddly enough little attention has been paid to myocardial injury with chemicals despite the relatively high incidence of heart disease in the general population. This is partially due to the indirect etiology of a significant fraction of human heart disease. However, the failure to detect the severe cardiotoxic effects of the anthracycline antibiotics in preclinical testing serves to illustrate the limited attention paid to this important organ in toxicity testing (Balazs and Ferrans, 1978).

Heart Failure Heart failure can be simply defined as inefficient operation of the heart as a pump. In the human disease this inefficiency is first reflected in hypertrophy, a compensated state. Eventually the gains in cardiac output which can be achieved by stretching the myofibrils is insufficient and heart failure results.

A variety of toxicological effects, could effect the efficiency at which the heart works. Some examples would include:

1. Interference with Ca^{++} entry upon depolarization
2. Interference with binding and/or release of Ca^{++} from intracellular sites.
3. Interference with Ca^{++} interaction with contractile apparatus
4. Impaired utilization of ATP by contractile apparatus.
5. Impaired energy metabolism
6. Functional uncoupling of energy metabolism i.e., leaky membrane.

The central role of cellular energetics in the myocardium is intuitively obvious because of the constant work done by the heart. The severe damage that can be done to the myocardium by relatively short periods of anoxia substantiates this fact (Kubler and Spieckermann, 1970). For this reason interference with cellular energetics and/or the utilization of ATP is very often

cited as the basis for heart failure, but rarely is there specific data to establish such hypotheses.

The best examples of chemically-induced heart failure is that produced by the anthracycline antiobiotics used in cancer chemotherapy. These chemicals are directly toxic to the myocardium. The chronic effects of these agents are quite complex including inhibition of RNA and protein synthesis, inhibition of Na-K-ATPase, inhibition of coenzyme Q reactions in cellular energetics, alterations in calcium transport, and promotion of lipid peroxidation (Balazs and Ferran, 1978). Given this diffuse picture, it is of interest that Cortes *et al.* (1978) have shown that administration of coenzyme Q to patients placed on adriamycin appears to prevent myocardial toxicity suggesting toxicity does arise from impaired energetics.

A second means of producing severe myocardial damage is through the exaggerated pharmacological effects of certain drugs. Beta-receptor agonists such as isoproterenol in high doses so stimulate the myocardium (via increased Ca^{++} influx) that oxygen supply to the myocardium is exceeded by demand produced by higher work level *and* mitochondrial accumulation of Ca^{++} (Fleckstein *et al.*, 1973; Balazs and Ferrans, 1978). This results in a severe subendocardial and papillary muscle necrosis.

With respect to direct myocardial damage there is little data upon which to judge the adequacy of various animal models. As mentioned earlier reasonably accurate reflection of anthracycline antibiotic activity is seen in the rat, mouse or rabbit (Balazs and Ferrans, 1978). Nevertheless, some caution is in order since the rat has long been known to react in an anomalous fashion to cardiac glycosides (Langer, 1978).

Conduction Anomalies Unlike the sparse reports of direct myocardial toxicity by environmental agents, there is a substantial literature linking halogenated solvents to cardiac arrhythmias. As previously indicated most of this literature has arisen from the occupational setting. Consequently, the major reason for the focus on these agents is the wide use of large quantities of these chemicals in industry. Therefore, it is difficult to determine if these properties are unique to this class of compounds or if this is the only class to be systematically investigated.

Detection of the arrhythmogenic activity of halogenated solvents in experimental animals has depended almost exclusively on one experimental model. Basically, this involves testing the effects of either short- or long-term exposures on the susceptibility of the myocardium to norepinephrine-induced arrhythmias. There has been fairly good agreement between agents that have been reported to produce arrhythmias in humans and those found positive in this test. However, the lack of information concerning other chemical classes makes it difficult to judge the adequacy of this testing procedure.

It would appear likely, however, that localized damage to the myocardium is frequently responsible for arrhythmias in humans (Rissanen, 1979; Brainwood, 1977). Such damage allows for the establishment of local circuits and ectopic foci that disrupt the normal excitation wave in the myocardium. It would seem, therefore, that a rational addition for testing arrhythmogenic potential of environmental chemicals would make use of the cardiomyopathic

Syrian hamster Bio 8262 strain (Stauch and Lossinger, 1973). This animal spontaneously develops focal necrotic myocardial lesions in the early stages of dystrophy, such lesions may hve the same arrhythmic potential as previous infarction has in sudden death victims. It is of interest to note that genetically cardiomyopathic hamsters have been shown to be as much as 5 times as sensitive to trichlorofluoromethane toxicity relative to random-bred hamsters (Taylor and Drew, 1975). Similar increases in sensitivity to this and related chemicals were observed in animals with pre-existing pulmonary vascular thrombosis or cardiac necrosis (Taylor and Drew, 1975). Consequently, this model could be very relevant to sensitive human populations.

SUMMARY AND CONCLUSIONS

Cardiovascular diseases account for more than 50% of the deaths in the U.S. As the major cause of death, measures or factors which have a relatively small effect on the development of these diseases have a much larger impact on the public health than more dramatic effects on less important diseases. The role environmental chemicals play i;n human diseases has become a popular cause in recent years, but has focused primarily on carcinogenesis, mutagenesis and teratogenesis. In the latter two cases at least, interest seems to be derived primarily from a general desire to prevent such occurrences, since it is not at all clear that substantial problems of these types exist in the environment (as opposed to industrial or occupational exposures). The pervasive nature of cardiovascular diseases seriously raises the question of whether a very important area has not generally been ignored in environmental health.

No answer presently exists to the question of whether a substantial fraction of human cardiovascular disease can be attributed to environmental chemicals. Nevertheless it is clear that chemicals derived from cigarette smoking do substantially increase coronary heart disease. While not as well established, the relationship between the chemical quality of drinking water and cardiovascular disease can be cited as another indication that some contribution of environmental chemicals to these diseases might exist. Finally, the potential for further effects can be visualized from the documented cardiovascular problems which have been encountered in industry and medicine.

Since cardiovascular diseases have long been the subject of bio-medical research, a substantial data base exists for a variety of experimental systems that are used to model different aspects of these disease processes. Most such systems have been developed for purposes of studying the progression of disease processes and for assessing the therapeutic value of various interventions. Toxicological studies using such systems have been almost entirely acute in nature. This concentration upon acute effects reflects the emphasis placed upon the cardiovascular area in toxicity testing by the pharmaceutical industry where the primary concern is in acute side effects of drugs used in short courses of therapy. Within the context of the area of acute toxicity testing, however, there are a variety of animal models which faithfully reproduce problems that have been encountered in humans with acute exposures to high concentrations of certain chemicals.

On the other hand, there is a general lack of basic information relative to the chronic effects of chemicals on the cardiovascular systems of experimental animals. Although well substantiated animal models for atherosclerosis, hypertension and other cardiovascular disease processes can be described, these systems have not been connomly employed in chronic toxicological studies. As a consequence there is no real basis on which to judge the appropriateness of different species for *toxicological* purposes. The obvious first step in cardiovascular toxicology is to establish through chronic experimentation whether representatives of environmental chemical classes do indeed increase cardiovascular diseases in the appropriate animal models. This would serve as a valid first step in assessing the potential of environmental chemicals to produce cardiovascular disease. Some results reported within this symposium indicate a start in establishing such a data base. The fact that some of these studies have taken their leads from human epidemiological studies will considerably increase their worth.

If the number of chemicals that pose a hazard for the cardiovascular system appears to be substantial, it is then important to design a testing strategy for addressing toxicity to the cardiovascular system. Fortunately, the cardiovascular system has been the subject of intense study by biomedical researchers for many years. As a result a very large variety of experimental systems (the vast majority of which have not been touched upon here) could be explored for their ability to predict chronic cardiovascular toxicity.

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